THE SPORES OF FOUR SPECIES OF SPINULOSE WOOD FERNS (DRYOPTERIS) IN EASTERN NORTH AMERICA

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In a recent paper Nannfeldt (1966) indicated that the diploid *Dryopteris assimilis* S. Walker could be readily distinguished from the tetraploid *Dryopteris dilatata* (Hoffm.) A. Gray by means of spore morphology and the color of the perispore. By using these characters, he was then able to accurately identify these species, and plot their distribution in Sweden. Earlier, Walker and Jermy (1964) emphasized that the spores of *D. assimilis* had a thin pale brown perispore with "widely spaced acute spinnules up to 1μ in length." The spore characters of various species of *Dryopteris* in North America were used by Crane (1963) to construct a key for identification. The data in this paper are presented to compare and contrast the spores of four species of spinulose wood ferns that are found in Eastern North America.

Although there is as yet no unanimity in the names used for these species, or indeed if they should be considered as species, workers are familiar with the names used here. The four taxa considered here as separate species consist of two diploids and two tetraploids. They are the diploid *D. intermedia* (Muhl.) A. Gray, a diploid segregate species allied to *D. dilatata* (Hoffm.) A. Gray which may be conspecific with *D. assimilis* S. Walker, *D. spinulosa* (O. F. Muell.) Watt *(D. carthusiana)*, and *D. campyloptera* Clarkson. *Dryopteris campyloptera* is considered by Wagner (1963) to be an amphidiploid of the first two species, whereas *D. intermedia* is part parental to *D. spinulosa* (Walker 1961). On the basis of cytological and morphological studies, all four species are closely related.

MATERIALS AND METHODS

Sori on mature fronds were gently scraped with a needle dipped in Permount in order to obtain spores. These spores

were then transferred to a drop of Permount on a slide and a cover glass was added. It was usually necessary to put a small weight on the cover glass in order to have a thin enough mount for observation with oil immersion. Measurements of exospore length were made using an ocular micrometer and a 90× apochromatic oil immersion objective. The calibration of the eye-piece was made with the aid of a micrometer slide. The calibration was carefully checked by an independent observer. One ocular division was equivalent to 1.4 microns. It is considered that due to operator error or idiosyncracy that the error in individual measurements is probably ± 1 ocular division or might be considered to be ca. ±1.5 microns.

Whenever possible, cytologically determined material was used for spore measurements. However, this proved impossible for four collections from Mt. Washington, N.H. of *D. campyloptera*, and for five collections of *D. dilatata* from near Lake Superior, Ontario. In these cases, it was necessary to rely on material that was morphologically similar to cytologically determined material from the same limited area, e.g. 12 collections of *D. campyloptera* had been studied cytologically from Mt. Washington (Britton 1962). Voucher Specimens are all at OAC except as indicated for five specimens designated by TRT: (Where no collector is given, the collection is by the author)

D. intermedia *620*, Conc. IV, lot 11, Puslinch Tp., Wellington Co., Ontario, 26 July 1962. *741*, *745*, *750*, *753*, Swan Lake, Algonquin Park, 20 July 1963. *825*, *826*, *843*, *853*, The Beaver Pond, Algonquin Park, Ontario, 15 August 1964. *861*, *H. M. Dale*, 2 mi N of Jefferson Notch near Mt. Washington, N.H. Alt. 2,300 ft., 8 Sept. 1964.

D. dilatata *715*, *717*, Garden transplants from N end of Jackfish Lake, Thunder Bay Dist., 25 June 1963. vouchers 23 August 1965. *854*, *900*, *901*. Garden transplants, *A. Asselin*, Lac Beaudoin, Amos, P.Q. 26 May 1965, vouchers 23 August 1965. (TRT 2, TRT 4), *Taylor, Bannan and Harrison*, Nos. *129*, *130*, Port Coldwell, vic. Peninsula, Thunder Bay Dist., 16 August 1939 (TRT). (TRT 6), *Hosie, Losee and Bannan*, No. *89*, Walker Lake, Schreiber, Thun-

der Bay Dist., 11 July 1937 (TRT). (TRT 7), *Taylor et al.*, No. *288*, Mamainse Mt., Algoma Dist., 18 July 1935 (TRT). (TRT 8), *Hosie, Losee and Bannan*, No. *91*, Slate Islands, Thunder Bay Dist., 30 July 1937 (TRT).

D. spinulosa *286*, *290*, Conc. VIII, Lot 20, Puslinch Tp., Wellington Co., Ontario, 22 August 1960. *299*, Conc. III, Lot 15, Puslinch Tp., Wellington Co., 19 August 1960. *305*, Conc. IV, Lot 11, Puslinch Tp., Wellington Co., 19 August 1960. *488*, Conc. IV, Lot 11, Puslinch Tp., Wellington Co., 28 Sept 1961. *504*, Conc. IV, Lot 14, Nelson Tp., Halton Co., 21 Sept 1961. *623*, *624* (see *620*) *716* (see *715* and *717*) *744* (see *741*, *745* etc.)

D. campyloptera *857-860* (see *861*) *971*, Garden Transplant, M. Landon, Yarmouth, N.S. 14 July 1965.

OBSERVATIONS

The average lengths of 20 exospores for each collection of ten plants of each of *D. intermedia*, *D. dilatata* and *D. spinulosa* and for five plants of *D. campyloptera* are given in Table 1. The extreme measurements for individual spores are shown as well as the overall mean for the species and the range of means of collections.

In Plate 1386, small sections of the perispore are illustrated to show the morphology of some of the spines present.

DISCUSSION

One of the initial decisions, was to decide how many spores of each collection to measure. The measurements of 10, 20, and 30 spores from each of five collections were analyzed by Dr. G. C. Ashton, Dept. of Mathematics. According to the analysis of variance made by him, 20 spores were a suitable number to measure. The 30 spore sample did not yield any further information.

The measurements, together with the morphology show that *D. intermedia* has the most distinctive spores of the four species. The spores of this species are smaller and are covered by long, narrow, sharp spines. The other three species cannot be easily identified by spore characters. These results are not in agreement with those of Wagner

TABLE 1 Lengths of exospores for four species of *Dryopteris*

Species	Collection	Mean length of 20 exospores in microns		Range of lengths in microns
D. intermedia	620	36.3		32-39
	741	34.4		34-39
	745	32.8		31-38
	750	32.2		29-35
	753	35.3		31-39
	825	35.2		28-39
	826	35.9		31-41
	843	36.9		31-42
	853	33.9		31-39
	861	30.9		28-34
	Mean	34.4	(31-37)	28-42
D. dilatata	715	37.4		34-41
	717	36.1		32-41
	854	37.1		34-42
	900	37.4		31-41
	901	36.8		34-41
	TRT2	39.6		35-48
	TRT4	37.8		34-42
	TRT6	37.8		35-39
	TRT7	36.3		34-42
	TRT8	37.1		35-41
	Mean	37.3	(36-40)	31-48
D. spinulosa	286	40.0		35-43
	290	39.8		35-43
	299	35.1		34-38
	305	39.4		36-43
	488	41.0		36-45
	504	36.8		32-42
	623	37.1		34-41
	624	37.0		34-39
	716	37.4		35-42
	744	39.3		36-43
	Mean	38.3	(35-41)	32-45
D. campyloptera	857	41.3		38-43
	858	40.6		36-46
	859	38.6		35-42
	860	39.9		35-45
	971	38.4		35-45
	Mean	39.8	(38-41)	35-46

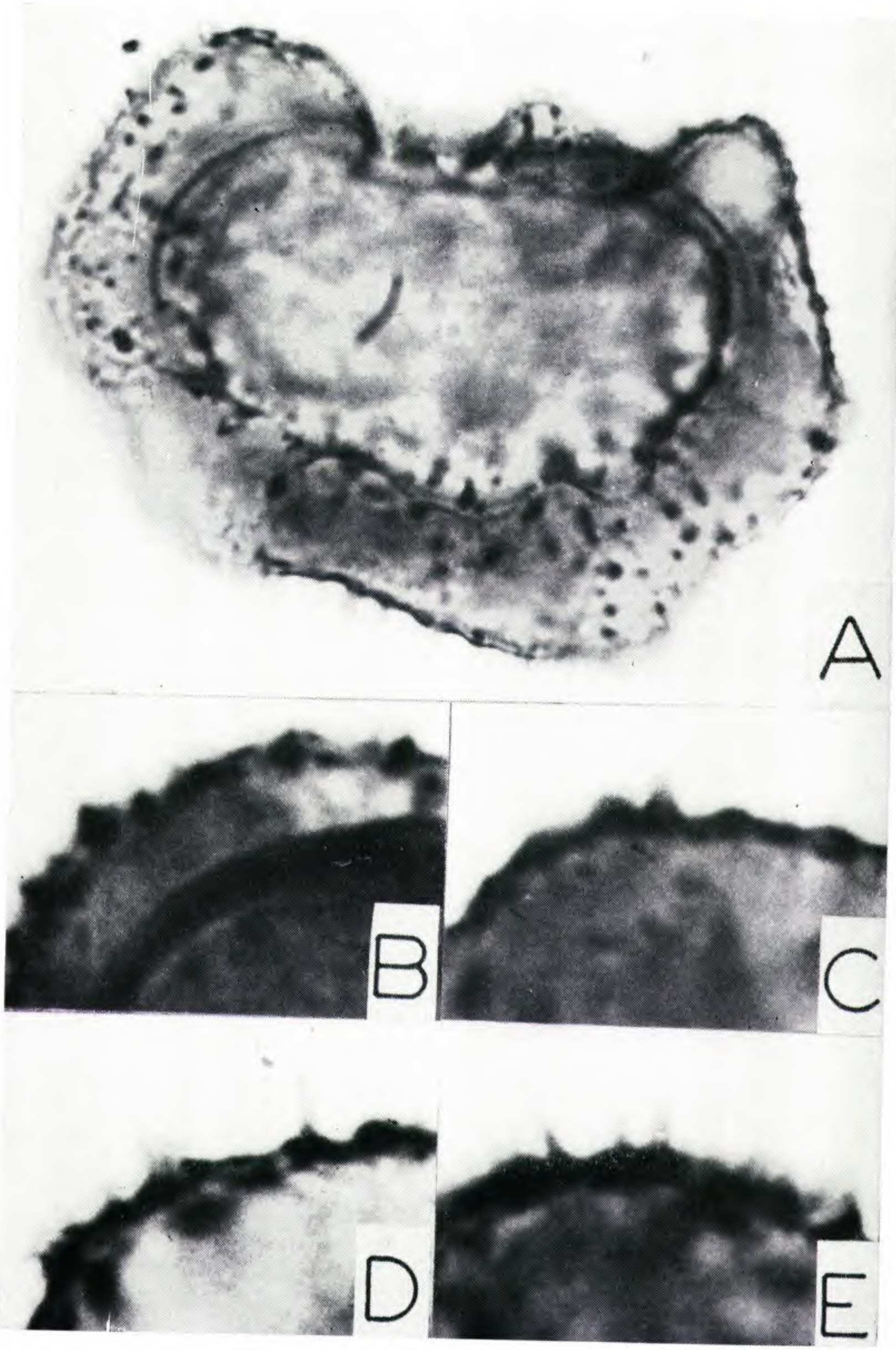

Plate 1386

A, Spore of *Dryopteris intermedia*, × 2000. B-E Portions of perispores to show the morphology of the spines, × 4700: B, *D. dilatata*; C. *D. spinulosa*; D, *D. intermedia*; E, *D. campyloptera*. Note the similarity between B and C and between D and E.

and Hagenah (1962) and Wagner (1963). My results are contrasted with those of these authors in Table 2.

TABLE 2 Mean exospore length and range of means in microns for four species of *Dryopteris*

	D. intermedia	*D. dilatata*	*D. spinulosa*	*D. campyloptera*
Britton	34.4 (31-37)	37.3 (36-40)	38.3 (35-41)	39.8 (38-41)
Wagner	32 (30-32)*	— (33-37)	—	38 (37-40)

*4 collections

As can be seen, Wagner's limited sample of four collections of *D. intermedia* from Virginia had smaller spores. The data are in good agreement for *D. campyloptera*, but differ markedly for *D. dilatata*. It was originally hoped that spore measurements would easily separate the diploid *D. dilatata* from the tetraploid *D. campyloptera*. Such is not the case. Although Wagner and Hagenah (1962) suggest that the averages for individuals of *D. dilatata* will be between 33-37 microns and for *D. campyloptera* from 37-40 microns my data do not support this contention. Eight of the ten collections of *D. dilatata* that were studied had an average exospore length (20 spores) of 37 or greater than 37 microns and none were smaller than 36 microns. Although the average size of all the collections of *D. campyloptera* is greater (39.8) than that of *D. dilatata* (37.3), this does not help in identifying single plants.

Crane (1960) has illustrated the spines of *D. spinulosa* as being blunt. In reality, all are sharp-tipped, but in morphology the sides of the spines are approximately as long as the base is broad, in contrast to the needle-like, narrow-based spines of *D. intermedia*. (Plate 1386)

The spines of *D. dilatata* are widely set and are more similar to those of *D. spinulosa* than to those of *D. intermedia* with its closely set, long, sharp spinnules. The spines of *D. campyloptera* can be visualized as showing the influence of both putative parents, *D. intermedia* and *D. dilatata*. The spines seem slightly longer and more pronounced than those of *D. dilatata* but they are more widely spaced than those of *D. intermedia*. However, the differences are not clear-cut, and it would be difficult to identify one species from another by perispore morphology alone.

Lovis (1964) has discussed the difficulties in obtaining accurate measurements for spore size in *Asplenium*. Calibration of the microscope, mounting medium, operator error and number of spores measured have been mentioned here. Besides these, there is the orientation of the spore on the slide, the possible unconscious selection of the largest spores, spores from sporangia produced late in the season and hence poorly developed, rather than from early sporangia from which the spores were shed. Also, spores from plants growing in very humid conditions versus those growing in drier locations and of course, genetic variability of the individual plants.

However, unlike *Asplenium trichomanes* (Lovis 1964), it would seem that spore size and ploidy are not closely correlated in *Dryopteris*. For example, *D. fragrans* is diploid and has large spores (Crane 1960), and in this paper, the diploid *D. dilatata* has spores which are approximately the size of those of the tetraploids *D. spinulosa* and *D. campyloptera*. Brown (1964) showed in *Woodsia* that the spores of three tetraploid taxa were not larger than those of closely related diploid species. In this respect, *Dryopteris* and *Woodsia* are similar.

CONCLUSIONS

The size of the spores as measured by the length of 20 exospores together with the ornamentation of the perispore allows one to identify diploid *Dryopteris intermedia*. *D. dilatata*, *D. spinulosa* and *D. campyloptera* cannot be easily separated by either spore size or perispore morphology. Accordingly, *D. dilatata* and *D. campyloptera* which have a similar leaf morphology cannot be easily identified. Hence, until further cytological studies are made, identification of *D. dilatata* (2×) and *D. campyloptera* (4×) for the purposes of plotting their distribution is not possible.

The critical cytogenetic evidence is still not available (Britton 1962) to decide the status of the taxon referred to as *D. dilatata*. However, the description of *D. assimilis* given by Walker and Jermy (1964) with particular emphasis on spore characters does not disagree with the spore

characters of the diploid segregate species referred to here as *D. dilatata*. The possibility exists that the diploid segregate species is conspecific with *D. assimilis*.

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